

OBSERVATION REPORT

A STANDARDIZED FRAMEWORK FOR QUANTITATIVE ANALYTICAL METHOD VERIFICATION TO ENSURE HIGH QUALITY REPORTING OF PATIENTS' SAMPLES IN A MEDIUM SIZED LABORATORY ASSOCIATED WITH TERTIARY CARE HOSPITAL

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Received: 30 January, 2020/ Revision: 21 February, 2020/ Accepted: 25 March, 2020

ABSTRACT: **Introduction:** Method verification and validation is an individual laboratory's responsibility in view of providing patient care with high reliability and as per requisite of regulatory bodies in health care domain. In this context, the study laboratory validates all new tests before introducing it for patient sample testing by verifying analytical accuracy, inter-assay and intra-assay precision, manufacturer's reference intervals and linearity range. **Materials and Methods:** The process of validation is being carried out using certified quality control material covering both normal and abnormal ranges of the analyte (Procalcitonin). Inter-assay and Intra-assay precision verification was performed wherein each level of the quality control material is processed 3 times per run for 5 days generating 15 replicates and 20 times in a single run respectively. Verification of Analytical Accuracy was expressed as recovery, calculated as the percentage between average measurement results and true value of the reference material. Reference Range has been verified by processing samples from 20 healthy individuals. For linearity check, high value sample above linearity range is progressively diluted, until it crosses lower limit of linearity and results expressed as recovery percentage. **Results:** Precision verification (inter and intra assay), accuracy, reference range and linearity verification were within the acceptable criteria defined by the laboratory (manufacturer's claim in case of procalcitonin). The test method was thus accepted to be used for patient sample testing. **Conclusion:** Method validation is an imperative part of maintaining laboratory tests quality. This study highlights the role of method validation as a vital step towards quality patient care. Individual laboratory can define its own method validation protocol in a cost-effective way.

KEYWORDS: Method validation, intra-assay precision, inter-assay precision, accuracy, linearity, reference range

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INTRODUCTION:

Method validation is pursued as the first step for all tests before being introduced for patient testing in a clinical laboratory set up. This guarantees maintenance of standard quality credentials and ensures that the laboratory test data and results are consistent, accurate and precise ^[1].

U.S. Food and Drug Administration defines validation as “Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.” ^[2]

When introducing an unmodified, US Food and Drug Administration–cleared or approved test system, laboratories need to verify manufacturer’s claim^[3]. Method verification is an abbreviated process to establish that a test performs in substantial compliance to previously established claims.

For validation of test methods and instruments used for analysis, laboratories should have well established protocols for system evaluation and qualification phases: Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ)^[2,4]. While IQ establishes that the equipment and its subsystems have been properly installed by the company’s representative, OQ validates that the instrument is operating according to the defined specifications. Performance Qualification ensures continued acceptable performance of an instrument under daily actual running conditions of the laboratory like temperature, humidity, electricity, operator skills etc. There are eleven main principles to the PQ laboratory test validation protocol which include specificity, linearity, accuracy, precision, robustness, range, limit of detection, limit of quantitation, ruggedness, selectivity, system and suitability, ^[5]

Method verification and validation studies is the responsibility of the laboratory in view of

providing patient care with a desired degree of reliability and as per requisite of professional and regulatory bodies in health care domain.

In this context, the study laboratory validates all techniques and new tests by verifying analytical accuracy, inter-assay and intra-assay precision, manufacturer’s reference intervals and linearity range, details of which are described below.

MATERIALS AND METHODS

The process of validation is being carried out using certified quality control material covering both normal and abnormal ranges of the analyte. In the current study, we have taken procalcitonin during the startup phase of the new instrument to enumerate all the validation steps discussed henceforth. Procalcitonin was estimated by immunoassay method based on sandwich principle on Cobas e411, a Roche Diagnostics platform.

The minimum studies performed in our laboratory for quantitative test system validation are

1. Precision Verification Study

- Inter-assay precision study (Intermediate Precision)
- Intra-assay precision study (Repeatability)

2. Verification of Analytical Accuracy

3. Verification of Reference Range

4. Verification of linearity and recovery

Carry over study is not recommended in case of Cobas e411 as separate microtip is used for assay of each patient sample.

Precision Verification Study

Precision implies repeatability, which means, analyzing a sample repeatedly to determine variation. Precision can be specified as: (i) repeatability (within run), (ii) intermediate

precision (long term) and reproducibility (interlaboratory)^[6].

To verify precision in the study laboratory, certified reference material covering both normal and abnormal ranges of the analyte (Elecsys BRAHMS Precicontrol, PCT 1 and PCT 2) are processed to determine repeatability and intermediate precision.

To determine intermediate precision or inter-assay variation, each level of the quality control material is processed 3 times per run for 5 days (as per CLSI protocol- EP15-A2), generating 15 replicates.

For determining repeatability or intra-assay variation, two levels of control material are run 20 times in a single run. Precision is quantified by calculating the mean, standard deviation (SD), and coefficient of variation (CV) of data collected from an analytical run.

The CV% obtained in the inter and intra assay precision study should be less than or equal to the manufacturer's claim.

Verification of Analytical Accuracy

Agreement between the test result and the true result was determined by the recovery of Certified Reference Material Value, traceable to a known standard.

For this, two levels of reference material spanning the entire analytical range of the test method to be validated were run in replicates of 20 in the same assay (intra assay run).

Accuracy is expressed as percentage recovery between average measurement results and the conventional true value which is the value of the certified reference material. It is considered to be acceptable if recovery is 100% (ideal) or between 90-110%

Verification of Reference Range

As per Principle of Transference of reference Intervals (CLSI C28-A2, Section 7), it is beneficial to be able to transfer a reference interval from one laboratory to another by process of validation which is less costly and more convenient^[7]. A laboratory can adopt reference limits from any of the following sources: manufacturer suggested, reference laboratory, published articles, neighboring laboratory or previous reference limits in the same laboratory^[5].

In the study laboratory, manufacturer's proposed limits were verified to satisfy that the reference ranges used by the laboratory are appropriate for the patients. For checking reference intervals, samples from selected 20 representative healthy individuals were processed, and the test was considered to be validated if ≤ 2 of them were outside the proposed limits.

Verification of Linearity and Recovery

For linearity check, an abnormal sample above the linearity range is selected and progressively diluted, until it crosses the lower limit of linearity. The expected value for each level of dilution is calculated. In case of Procalcitonin, the measuring range is 0.02-100 ng/ml. Patient sample with a value of 184ng/ml was selected to verify linearity of the method.

The samples were processed in a sequence from undiluted sample to the sample having highest dilution and the observed values for each level of dilution is noted.

Verification of linearity is considered acceptable if the percentage recovery is within an acceptable limit 90-110%, where ideal recovery of any test is 100%.

RESULTS AND OBSERVATIONS:

The validation results of procalcitonin are described sequentially below.

Precision Verification study

Intra-assay and Inter-assay precision verification was found to be within the acceptability criteria defined by the study laboratory ($CV\% \leq$ manufacturer's claim).

Table 1 and Table 2 show the results obtained for inter-assay and intra-assay precision verification for both levels of quality control.

Table 1. Inter-Assay (Intermediate) Precision

Inter-Assay Precision (ng/ml)		
	Level 1	Level 2
DAY 1	0.49	10.1
	0.50	10.1
	0.50	10.3
DAY 2	0.49	10.3
	0.50	10
	0.50	10
DAY 3	0.50	10.3
	0.51	10.2
	0.51	10.2
DAY 4	0.48	10
	0.48	10.1
	0.49	9.9
DAY 5	0.49	10
	0.49	9.9
	0.48	9.8
Number of runs	15	15
Mean	0.49	10.08
Standard Deviation	0.01	0.16
Observed CV%	2	1.56
Manufacturers CV%	3.7	4.0

Inter-assay precision verification run in replicates of three for five days shows acceptable results as the observed CV% for both levels of quality

control is less than the CV% obtained by the manufacturer.

Table 2. Intra-Assay (Repeatability) Precision

Intra-Assay Precision (ng/ml)		
Replicates	Level 1	Level 2
1	0.49	10.1
2	0.49	10.1
3	0.50	10
4	0.50	10
5	0.49	10.1
6	0.49	10
7	0.50	10
8	0.49	10
9	0.50	10.1
10	0.49	10.1
11	0.48	10.1
12	0.49	10
13	0.50	9.9
14	0.50	10.1
15	0.49	9.9
16	0.49	10
17	0.49	10
18	0.50	9.9
19	0.49	10.1
20	0.49	10.1
Mean	0.49	10.03
Standard Deviation	0.01	0.07
Observed CV%	1.16	0.73
Manufacturer's CV%	1.3	0.9

Intra-assay precision to determine repeatability of the test was performed wherein 20 replicates of both levels of quality control material were processed. The results obtained were acceptable as the observed CV% was less than the manufacturer claimed CV%.

Verification of Analytical Accuracy

Analytical accuracy of the test was elucidated by the recovery obtained after both the quality control materials were run in replicates of 20 in the same assay (intra assay run). Recovery has been calculated as percentage between the average measured results and the conventional true value of the reference material.

Accuracy, as determined by recovery was found to be within acceptable limits for level 1 and level 2 controls, which was 100% and 101% respectively. This ensured closeness of the observed value (L_1 -0.49 ng/ml; L_2 -10.3 ng/ml) to the actual mean value (L_1 - 0.49 ng/ml; L_2 -10.1 ng/ml) of the reference material.

Verification of Reference Range

The study laboratory verified the manufacturer's proposed limits for the reference range of the test method used by processing samples from selected 20 representative healthy individuals.

The Table 3. below describes the results obtained from reference range verification study.

Table 3. Verification of Reference Range

Serial No.	Values
Sample 1	0.41
Sample 2	0.46
Sample 3	0.48
Sample 4	0.4
Sample 5	0.42
Sample 6	0.4
Sample 7	0.5
Sample 8	0.49
Sample 9	0.46
Sample 10	0.46
Sample 11	0.42
Sample 12	0.53
Sample 13	0.46
Sample 14	0.49

Sample 15	0.44
Sample 16	0.45
Sample 17	0.42
Sample 18	0.46
Sample 19	0.47
Sample 20	0.47
Mean	0.45
SD	0.04
Reference Range Obtained	0.41-0.49
Manufacturer's Reference Range	≤ 0.5

The reference range obtained by the study laboratory for the test method is 0.41-0.49 ng/ml. The values obtained from all the 20 samples from selected healthy individuals were within the proposed limits by the manufacturer. Manufacturer's reference range was thus verified and was adopted for reporting patient results by the laboratory.

Verification of Linearity and Recovery

Patient sample above the linearity range for the test method was selected and progressively diluted, until it crossed the lower limit of linearity. Recovery value for each level of dilution was calculated. For Procalcitonin, the measuring range is 0.02-100 ng/ml. Patient sample with a value of 184ng/ml was selected to verify linearity of the method.

Table 4. below shows the results obtained for linearity verification of the test method.

Table 4. Verification of Linearity and Recovery

S No.	Dilution	Observed Value	Expected Value	Recovery (%)
1	0	184	-	-
2	1:400	0.42	0.46	91.3
3	1:800	0.24	0.23	104.3
4	1:1500	0.12	0.12	100
5	1:3000	0.059	0.06	98.3
6	1:6000	0.032	0.03	106.6

7	1:8000	0.02	0.02	100
	Mean Recovery %			100.08
	Standard deviation			5.3
	Percentage Recovery Range			94.78-105.38
	CV%			5.3

Linearity verification was found to be adequate with recovery percentage range of 94.78-105.38, falling within the acceptable criteria (90-110%) set by the laboratory.

DISCUSSION:

Though an extensive validation ensures dependability on the test, every laboratory can decide upon the same while taking care of their patient sample load, cost effectiveness, type of equipment's etc. In this literature, we tried to elaborate the study laboratory's essential method validation steps before introducing new tests for patient sample testing. This included verifying the analytical accuracy, precision, manufacturer's reference range, verification and linearity.

Acceptability criteria for successful validation were defined by the laboratory to be one-third of the total allowable error for each analyte, source of which was taken from different industry standards (eg. Clinical Laboratory Improvement Amendments, College of American Pathologists, Ricos biological variability goals etc.) The choice of the source of total allowable error was based on the fact that it should be adequate to avoid false rejections and at the same time should not miss out on potential errors.

In the absence of documented total allowable error for an analyte, acceptability goal of validation was taken to be less than or equal to the values obtained by the manufacturer.

For procalcitonin, in the current study, the acceptability criteria were taken to be less than or equal to the manufacturer's CV% claim. All steps

of method validation (precision verification, accuracy verification, manufacturer's reference range verification and linearity verification) for the analyte of interest fulfilled the acceptability criteria defined by the laboratory and thus approved the test method to be used for patient sample testing.

If the acceptability criteria are not met, the cause should be evaluated and validation should be repeated after implementing corrective actions.

The fundamental role of any clinical laboratory is the desire to report precise patient results. In the era of evidence-based medicine, the quality of evidence must be verified (8) and validation of methods is an imperative part of that process.

As a crucial step towards quality patient care, the study laboratory does systematic validation of all new tests to warrant high reporting standards. The laboratory also has written-down standard operating protocols for validation of new tests to ensure uniformity of the process.

CONCLUSION:

Test method validation is an imperative part of maintaining laboratory tests quality and ensuring high reporting standards consistent with patient's clinical findings. This study highlights the role of method validation as a vital step towards quality patient care. Individual laboratory can define its own method validation protocol in a cost-effective way. It is also of utmost importance for every laboratory to design a standard operating procedure for method validation to ensure uniformity of the process across all new tests.

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Cite of article: Datta RR, Sharma LK, Srivastava M, Sharma N. A standardized framework for quantitative analytical method verification to ensure high quality reporting of patients' samples in a medium sized laboratory associated with tertiary care hospital. Int J Med Lab Res. 2020;5(1):41–47. <http://doi.org/10.35503/IJMLR.2020.5107>

CONFLICT OF INTEREST: Authors declared no conflict of interest

SOURCE OF FINANCIAL SUPPORT: Nil

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